# BABA GHULAM SHAH BADSHAH UNIVERSITY

## RAJOURI

SYNOPSIS FOR Ph.D REGISTRATION

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| PROPOSED RESEARCH TOPIC | : | Systematic and Ecological studies of Rhabditid nematodes of District Rajouri. |
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| NAME OF THE SUPERVISOR | : | Dr. Ali Asghar Shah |
| SCHOOL/DEPARTMENT | : | BIO-SCIENCES & BIO-TECHNOLOGY |
| SUBJECT | : | ZOOLOGY |
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Signature of the Candidate

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**Background**

Free living nematodes are the soil fauna that play a key role in mediating soil nutrients by feeding on fungi, bacteria, cyanobacteria, green algae, plant roots and other soil fauna (Bardgett *et al*,. 1999; Whitford, 1996). Nematodes are used as indicators of the structure and function of the soil community (Nehar and Campbell, 1996; Neher *et al*., 1998) because they represent multiple trophic groups and are ubiquitous, intimately linked to the soil environment, and sensitive to environmental changes (Neher, 2001).

Nematodes, popularly known as roundworms, are the most numerous organisms on earth. They surpass all the organisms not only in number but in their species diversity also. Four of every five multicellular animals on the planet are nematodes (Gunapala *et al*. 1998). Their distribution extends from deep ocean trenches to the top of high mountains covering all possible habitats including hot springs, ice, low oxygen, acidic environment, etc. Some of them also can withstand complete dryness on the surface of rocks. Their size too is extremely variable ranging from less than 100 µm *(Greefiella minutum*) to greater than 8 m (*Placentonema gigantissma).* Their abundance reaches many million individuals per $m^2$ in soil and bottom sediments of aquatic habitats and a handful of soil may contain more than 50 species.Many species are important parasites of plants and animals including human beings, while a large number of species are free-living , aquatic or terrestrial.

Knowledge of animal parasitic nematodes is very ancient and dates back to about 1,500 B.C. when the dreaded Guinea worm, *Dracunculus medinensis* and the large round worm, *Ascaris lumbricoides* were known. However, marine, freshwater, soil and plant parasitic

nematodes remained little known groups mainly because of their extremely small size and the difficulties encountered in their isolation, mounting and observation, Borellus (1656) for the first time observed the vinegar eel worm, *Turbatrix aceti* and Needham (1743) was the first to observe a plant parasitic nematode, *Anguina tritici* in the cockles of wheat when he examined a portion of the crushed cockle under his primitive microscope. Steinbuch (1799) named them *Vibrio tritici*.

It was only in the middle of the nineteenth century that work on the free-living nematodes was started in earnest. Dujardin (1845) first established the genus *Rhabditis* with *Rhabditis terricola* as its type species. However, it was not clearly defined until more than one hundred years later (Dougherty, 1955), Orley (1880) tried to fit the genus *Rhabditis* into the system of nematoda and proposed a family Rhabditidae for the genera *Anguillula, Cephalobus*, *Oxyuris, Rhabditis* and *Teratocephalus* and placed this family in the higher category 'Rhabditiformae' which formed a connecting link between free-living and animal-parasitic nematodes. He provided new information regarding biology and taxonomy of *Rhabditis* besides a key for the identification of species. De Man (1876, 1880and 1884) added many new species raising the total number to 37 in this genus Micoletzky (1922) in his monograph on the non-marine free-living nematodes listed 142 valid genera and 931 species. Cobb (1913) proposed the genus *Diploscapter* for *Rhabditis coronata*. Goodey (1963) accepted four subfamilies: Alloionematinae, Protorhabditinae, Diploscapterinae and Rhabditinae under Rhabditidae and also proposed Pterygorhabditinae under Bunonematidae.

Andrassy (1976) accepted three superfamilies viz., Alloionematoidea (Chitwood and McIntosh, 1934), Rhabditoidea (Orley, 1880), and Bunonematoidea ( Micoletzky, 1922), under

Rhabditina. Under Rhabditidae he proposed seven subfamilies including three new ones viz., Mesorhabditinae, Peloderinae and Ablechroiulinae and upgraded the subfamily Pterygorhabditinae to family Pterygorhabditidae under Bunonematoidea. Sudhaus (1976) accepted the genus *Rhabditis* and placed fifteen subgenera under it where, *Xylorhabditis* was newly proposed, but did not accept the subfamilies Protorhabditinae (Dougherty, 1955), Prodontorhabditinae (Timm, 1961). and Parasitorhabditinae (Lazarevskaja, 1955). and placed *Protorhabditis, Prodontorhabditis* and *Parasitorhabditis* in Rhabditinae. Under Rhabditina (Andrassy, 1983). accepted three superfamilies, seven families and fourteen subfamilies and he included two new genera viz., *Dolichorhabditis* and *Rhomborhabditis* under the subfamily Peloderinae. (Sudhaus, 1976) gave *Xylorhabditis*generic rank and in Rhabditinae the genera *Discoditis* and *Rhitis* were proposed.

One pertinent tool for ecological analysis of nematodes is the maturity index (MI) to indicate the successional maturity of nematode communities and, thus, the biological condition of the soil habitat (Bongers, 1990). Nematodes are assigned a colonizer persister value (or c-p value) ranging from enrichment colonizers (c-p = 1) and disturbance colonizers (c-p = 2) to persisters (c-p = 5); the MI is the weighted mean for the frequency distribution of collective c-p value. Colonizers generally exhibit a relatively short lifespan and high fecundity, while persisters are often longer-lived and slower to reproduce (MacArthur and Wilson, 1967; Grime, 1979). Accordingly, soil faunal communities with ample nutrients, but lacking disturbance or eutrophication, are assigned greater MI value than do recently or continually disturbed communities.

Because of the free-living nature, these nematodes are found to be sensitive to slight environmental changes and this sensitive nature makes them a good bioindicator. These nematodes may also serve as good laboratory models for some biochemical and molecular analysis.

There are apparently significant possibilities for the use of nematode populations as bioindicators for all soil conditions. However, despite universal occurrence and evident importance in some ecosystems the taxonomic composition and functional role of these nematodes is largely unknown.

In India, the work on free-living nematodes was not carried out extensively. Gaining some taxonomic value the workers started working on these nematodes but still most of the free-living fauna from India are largely unknown.

As far as nematode fauna of district Rajouri is concerned, there has never been a thorough survey. This gives clear indication of the paucity of information on the nematode fauna of this District and hence justifies the proposed study.

Morphology has been and will continue to be the mainstay of nematode taxonomy. It is an essential component of any higher level classification of nematodes and in many cases morphology provides a rapid and unambiguous diagnosis to species. However, the identification of intrasubspecific groupings requires the application of other taxonomic techniques such as DNA sequence analysis. In view of the above DNA-based taxonomic methods will be used for the identification of nematode species wherever required.

The present plan of work is proposed to investigate the ecology and diversity of Rhabditid fauna of different habitats of district Rajouri.

**Aims and objectives**:

- Make an in-depth systematic study of nematodes.
- Make a detailed ecological analysis of the nematodes to understand the soil ecology of District Rajouri using ecological parameters.
- Study functional diversity of nematodes of District Rajouri so as to compute structure index (SI) and Enrichment index (EI).
- To check the organic and inorganic contents of the collected soil samples of district Rajouri.
- Molecular characterization of the isolated taxa using RAPD markers wherever required.

**Methodology**

**(a) Methodology for Ecological study**

**Sampling**: Soil samples will be collected in different seasons from different regions of District Rajouri. Each sample (500 g) will be representative of a single excavation of soil from 15 cm diameter and 30 cm deep. The samples will be kept in air tight polythene bags. All relevant information's such as locality, date of collection, etc, will be noted and the samples brought to the laboratory for further processing.

**Study of soil:**

**Soil pH:** The pH will be determined using pH meter on a 1:1 suspension (5 gram scoop of soil to 5 milliliters water).

**Soil moisture**: The moisture content of the samples will be determined by oven-drying method.

**Soil organic matter:** Organic matter will be determined by ashing a 5 gram scoop of the soil sample at 360$^{O}$C for 2 hours. The loss by weight of the sample during this ignition is calculated as the organic matter.

**Nematode enumerations:** Nematodes will be extracted from soil using a modified Cobb's decanting and sieving and modified Baermann's funnel techniques. Nematodes will be counted, heat relaxed, fixed in hot F.A. (4:1), and mounted on temporary slides for identification of at least 10% or up to 150 representative individuals per sample to genus level according to Thorne (1974), Jairajpuri and Ahmad (1992), Hund (1993), Bongers (1994), Siddiqi (2000), and De Ley *et al*., (2003). Nematodes will be assigned trophic groups according to Yeates *et al*., (1993a) and colonizer-persister groups based on Bongers (1990).

**Community indices**: Nematode communities will be characterized using six indices. Generic richness defined as the total number of genera observed per sample. Hill's diversity index will be calculated as $N_1 = \exp(-\Sigma p_1 \ln(p_1+0.001)$ where $p_1$ is the proportion of each genus i (Hill, 1973) . Maturity index (Bongers, 1990) a measure of nematode community succession, will be calculated as $MI=\Sigma (v_i p_i)$,where $v_i$, is the colonizer-persister (c-p) value assigned to free-living (not berbivorous) taxa I, and pi is the proportion of taxon I, The Plant Parasite Index, an ecological maturity index of herbivores, will be calculated as $PPI= \Sigma (v_i p_i)$ as in the MI but where taxa I include only herbvivorous nematodes (Bongers, 1990). Enrichment index (EI) and Structure index (SI) will be calculated according to Ferris *et al*., (2001).

**Statistical analysis**: Three types of statistical procedures will be performed to characterize associations among nematode genera and environmental properties. First, analysis of variance (ANOVA) will be performed to determine if the proportion of nematode genera differed

between location and crust type. This analysis will be restricted to observations made in the top 10 cm of soil because these nematodes are most likely to interact with crust as food and or habitat. Location, crust type, and the two-way interaction between location and crust are main effects, and proportions of genera are dependent variables.

**(b) Methodology for Taxonomic Study:**

For morphological identification the methodology involve following steps.

**Isolation of nematodes**: Nematodes will be isolated by using modified Cobb's sieving and decantation and modified Baermann's funnel technique.

**Killing and fixation of nematode**: The nematodes will be killed and fixed in hot FA 4:1 (Seinhorst, 1962).

**Dehydration, mounting and sealing**: After fixation the nematodes will be transferred into a mixture of glycerine-alcohol (5 parts glycerine+ 95 parts 30% alcohol). Gradual dehydration will be done using anhydrous calcium chloride. Mounting will be done in anhydrous glycerin and sealing using wax.

**Measurements and drawings**: The measurements will be taken by using an ocular micrometer and dimensions will be expressed by using de Man's formula. The illustrations will be drawn with the help of a drawing tube attached to Olympus microscopes.

**Photography**: Photographs will be taken by using a digital camera attached to Olympus microscope.

**Molecular Taxonomy**

Nematodes will be cultured and maintained in agar medium till their final use. Nematode extraction buffer (NEB) method proposed by Z. A. Handoo *et.al.*, with modifications wherever

required shall be followed for genomic DNA isolation. After purification, the extracted worm DNA shall be subjected to RAPD marker analysis.

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